

Claims

1. A method to prepare a desired polyketide synthase from individual modules which method comprises
providing successive covalently linked modules that comprise an intrapolyptide linker (RAL) and successively non-covalently linked modules that comprise interpolyptide linkers (ERL), so as to facilitate the transfer of a nascent polyketide chain from the a module of said PKS to a succeeding module of said PKS; and
assembling said modules.

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10 2. The method of claim 1 wherein said assembling is by incubating the polypeptides which comprise said modules in a reaction mixture.

15 3. The method of claim 1 wherein said assembling is by expressing constructs which encode said modules in a host cell.

20 4. The method of claim 1 wherein at least some of the modules in the desired polyketide synthase are derived from a library of Type I PKS modules and wherein at least one module in said polyketide synthase is heterologous with respect to the remaining modules.

25 5. The method of claim 1 wherein each RAL has an amino acid sequence selected from the group consisting of those set forth in Figure 3 or a variant thereof and the N-terminal portions of each ERL has the amino acid sequence set forth in Figure 3 or a variant thereof.

6. A polyketide synthase prepared by the method of claim 4.

7. The polyketide synthase of claim 6 which contains erythromycin modules 1, and 3-6 and tylosin module 2, and wherein said polyketide chain is transferred from *ery* module 1 to *tyl* module 2 and then to *ery* modules 3-6.

5 8. The polyketide synthase of claim 6 which contains erythromycin modules 1-5 and narbomycin module 6, wherein said polyketide chain is passed from *ery* modules 1-5 to *nar* module 6.

10 9. The polyketide synthase of claim 6 which contains modules 1 and 3-6 of erythromycin and modules 2-3 of tylosin, spiramycin or niddamycin, wherein said polyketide chain is passed from *ery* module 1 to modules 2-3 of tylosin, spiramycin or niddamycin and then to *ery* modules 3-6.

15 10. The polyketide synthase of claim 6 which contains modules 1-3 of tylosin, spiramycin or niddamycin and modules 3-6 of erythromycin, and wherein said polyketide chain is passed from modules 1-3 of said tylosin, spiramycin or niddamycin to *ery* modules 3-6.

20 11. The polyketide synthase of claim 6 which contains a module of tylosin, spiramycin or niddamycin and modules 1-2 and 3-6 of erythromycin, wherein said polyketide chain is passed from *ery* modules 1-2 to the tylosin, spiramycin or niddamycin module and then to *ery* modules 3-6.

25 12. The polyketide synthase of claim 6 which contains modules 1 and 3-6 of erythromycin and module 5 of tylosin, spiramycin or niddamycin having the enoyl reductase catalytic activity inactivated, wherein said polyketide chain is passed from *ery* module 1 to module 5 of tylosin, spiramycin or niddamycin and then to *ery* modules 3-6.

30 13. The polyketide synthase of claim 6 which contains erythromycin modules 1-4 and 6 and module 6 of spiramycin or niddamycin, wherein said polyketide

chain is passed from *ery* modules 1-4 to module 6 of spiramycin or niddamycin and then to *ery* module 6.

5 14. The polyketide synthase of claim 6 which contains module 1 of FK-506/520 and modules 2-14 of rapamycin, wherein said polyketide chain is passed from module 1 of FK-506/520 and then to modules 2-14 of rapamycin.

10 15. The polyketide synthase of claim 6 which contains module 1 and 11-14 of rapamycin and modules 2-6 of FK-506/520 wherein said polyketide chain is passed from module 1 of rapamycin to modules 2-6 of FK-506/520 and then to modules 11-14 of rapamycin.

15 16. The polyketide synthase of claim 6 which contains module 1 of rapamycin, modules 2-7 of FK-506/520 and modules 12-14 of rapamycin, wherein said polyketide chain is passed from module 1 of rapamycin to modules 2-7 of FK-506/520 and then to modules 12-14 of rapamycin.

20 17. The polyketide synthase of claim 6 which contains module 1 of rapamycin, modules 2-8 of FK-506/520 and modules 13-14 of rapamycin, wherein said polyketide chain is passed from module 1 of rapamycin to modules 2-8 of FK-506/520 and then to modules 13-14 of rapamycin.

25 18. The polyketide synthase of claim 6 which contains modules 1-10 of rapamycin and modules 7-10 of FK-506/520, wherein said polyketide chain is passed from modules 1-10 of rapamycin to modules 7-10 of FK-506/520.

19. A method to prepare a desired polyketide which method comprises incubating required substrates with the polyketide synthase of claim 6.

20. The method of claim 19 wherein the substrates comprise a diketide thioester and thioesters of the required extender units.
21. The method of claim 20 wherein the extender units are malonyl, methylmalonyl, ethylmalonyl or hydroxymalonyl thioesters.
22. A polyketide or polyketide derivative synthesized by a method comprising the method of claim 19.

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